Persistence and biodegradation of vinclozolin in tropical rice soils

Anjana Banerjee, Sanhita Padhi and Tapan K Adhya*
Laboratory of Soil Microbiology, Division of Soil Science & Microbiology, Central Rice Research Institute, Cuttack 753006, India

Abstract: The persistence of vinclozolin in three tropical rice soils, widely varying in their physicochemical characteristics, was compared under both non-flooded and flooded conditions. Degradation of the fungicide was more rapid in all the soils under flooded conditions than under non-flooded conditions. Kinetic analysis indicated that the degradation of the fungicide followed a first-order reaction irrespective of soil or water regime. Soil acidity and salinity significantly affected the persistence of the fungicide under non-flooded conditions. The degradation of the dicarboxymide fungicide was enhanced following repeated applications to an alluvial soil under both water regimes, with the enhancement being more marked under flooded conditions. Faster degradation of vinclozolin in mineral salts medium inoculated with non-sterile suspension from retreated alluvial soil indicates the involvement of micro-organisms. 3,5-Dichloroaniline was detected as a metabolite in the degradation of the fungicide in both soil and mineral salts medium.

1 INTRODUCTION

Use of pesticides has ensured sustained high yields of man's agricultural products including food and fibres by protecting the modern high-yielding cultivars from disease attack. Flooded rice soils, in view of their dynamic aerobic–anaerobic interface, result into biodegradation of several recalcitrant pesticides.[1] Otherwise-persistent organochlorine pesticides have been shown to be readily degraded in flooded soils either unplanted or planted to rice.[2] Some of these organochlorine compounds, including recalcitrant isomers of hexachlorocyclohexane (HCH), have been shown to undergo enhanced degradation in flooded soils upon repeated application of the parent compounds[3] and or their metabolites.[4]

The dicarboxymide fungicide vinclozolin [(*RS*)-3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione] is used to control diseases caused by *Botrytis* sp, *Alternaria* sp, *Sclerotinia* sp and *Monilia* sp of vegetables and other field crops.[5] The fungicide undergoes hydrolysis and produces several degradation products, including 3,5-dichloroaniline.[6–8] About 50% of the fungicide is reported to be degraded in aerobic soils within 23 days and the degradation is rather slow in acidic soil.[9] We have studied the persistence and biodegradation of this dicarboxymide fungicide in three tropical rice soils under flooded and non-flooded conditions. Also, the development of accelerated degradation of vinclozolin in a flooded soil upon its repeated application has been investigated.

2 MATERIALS AND METHODS

2.1 Soils

Three soils from rice-growing areas of India, widely varying in their physicochemical characteristics (Table 1), were used in the study. The soils were air-dried and ground to pass through a 2-mm sieve before use.

2.2 Fungicide

For persistence studies under laboratory conditions and for greenhouse studies, a commercial vinclozolin 500 g kg^{-1} WP ('Ronilan' BASF, Germany) was used. For degradation studies in culture medium, analytical grade vinclozolin (99%; BASF, Germany) was used.

2.3 Soil incubation studies

Portions (20 g) of each soil were placed in sterile test tubes (220 × 25 mm) and moistened with sterile distilled water to maintain them at 60% moisture-holding capacity (for the non-flooded system) or flooded with 25 ml sterile distilled water (for the flooded system). After incubation for 10 days at room temperature (28(± 4) °C), an aqueous suspension of the commercial formulation of vinclozolin was added to the flooded or non-flooded soils to provide a final concentration of 50 μg AI g^{-1} soil. Following addition of the fungicide, the tubes were incubated at room temperature in the dark. Loss of moisture during incubation was compensated by adding the required quantity of sterile distilled water at weekly intervals. At periodic intervals, the residues of the fungicide in the

* Correspondence to: Tapan K Adhya, Laboratory of Soil Microbiology, Division of Soil Science & Microbiology, Central Rice Research Institute, Cuttack 753006, India
E-mail: crri@x400.nicgw.nic.in
Contract/grant sponsor: Department of Science and Technology, Government of India.
Contract/grant sponsor: University Grants Commission, New Delhi.
(*Received 17 February 1999; revised version received 13 July 1999; accepted 6 August 1999*)

 Pestic Sci 0031–613X/99/$17.50

Table 1. Physicochemical characteristics of the soils used in the study

Soil	Taxonomic group	pH	Organic matter (%)	Total N (%)	SO_4 (mg kg^{-1})	Electric conductance (dS m^{-1})	CEC (meq g^{-100} soil)	Soil separates		
								Clay (%)	Silt (%)	Sand (%)
Alluvial	Haplaquept	6.16	0.82	0.09	10.20	0.51	9.55	25.90	21.60	52.50
Acid sulfate saline (Kari)	Sulfaquept	3.41	24.61	0.37	321.64	6.93	28.91	29.40	6.80	63.80
Acid sulfate saline (Pokkali)	Sulfaquept	5.40	5.51	0.21	543.20	3.20	19.20	45.60	7.80	46.60

soil were analysed by gas liquid chromatography following extraction and clean-up.

2.4 Accelerated degradation in retreated soils under greenhouse conditions

Accelerated degradation of vinclozolin was studied under greenhouse conditions as described by Bharati *et al.* [4] An aqueous suspension of vinclozolin WP was applied at 10 μg AI g^{-1} soil to 5 kg of the alluvial soil contained in earthenware pots (25.5 × 9.5 cm). Two series of pots (unplanted and planted to rice) were maintained under four treatments *viz* (1) pots retreated with vinclozolin and maintained under non-flooded condition, (2) pots retreated with vinclozolin and maintained under flooded condition, (3) untreated control pots maintained under non-flooded conditions and (4) untreated control pots maintained under flooded conditions. Twenty-one-day-old rice seedlings (*Oryza sativa* L, cv CR 749-20-2) planted in pots represented the planted series. All the pots were maintained in the greenhouse under appropriate water regime with 60% moisture-holding capacity for non-flooded pots and with standing water of 5 (± 1) cm above the soil for flooded pots. The moisture level of non-flooded and flooded pots was maintained by weekly additions of the required quantity of water. The first application of vinclozolin was made to the soil in the pots at 10 days after flooding (unplanted pots) or transplantation (planted pots). A second application was made 15 days later (25 days after flooding) and subsequent applications were made at 40 days (50 days after flooding) and 55 days (65 days after flooding).

At regular intervals, soil samples from pots treated as above were collected as described by Panda *et al*[10] and tested for vinclozolin degradation. In brief, soil samples from the surface layer (1–2 cm) of pots from both sets (unplanted and planted) were collected separately by using a tube auger with a mark at 2 cm. Portions (1 g) of the soils thus removed were used to prepare suspensions in sterile distilled water (10 ml) in pre-sterilized test tubes (200 × 25 mm). Portions (10 ml) of sterile mineral salts medium [$(NH_4)_2HPO_4$ 0.5; $MgSO_4$, $7H_2O$ 0.2, $FeSO_4$, $7H_2O$ 0.01, K_2HPO_4 0.1, $Ca(NO_3)_2$ 0.01 g $litre^{-1}$ in distilled water; pH 7.0] contained in 100-ml Erlenmeyer flasks and supplemented with 10 μg ml^{-1} of vinclozolin were separately inoculated with 1 ml of soil suspension from both planted and unplanted pots. The flasks were incubated under aerobic conditions in a shaker at room temperature (28 (±2) °C). Uninoculated medium served as control. At periodic intervals, 1–2 ml portions of the inoculated and uninoculated media were drawn aseptically from each duplicate set and the amount of vinclozolin remaining in the medium was determined by gas-liquid chromatography after extraction with solvents. 3,5-Dichloroaniline, the degradation product of vinclozolin was detected in both soil and mineral salts medium by production of diazo colour complex[11] but was not quantified further.

2.5 Extraction of residues

In the experiment on the persistence of vinclozolin in different soils, residues of the parent fungicide were extracted from the soil. Soil plus water in each of the duplicate tubes was quantitatively transferred to a 250-ml Erlenmeyer flask with 20 ml distilled water and 50 ml acetone. After 2 h equilibration on a rotary action shaker, 20 ml hexane was added. After shaking for another 1 h, the volume was adjusted to 250 ml with 20 g $litre^{-1}$ aqueous sodium sulfate. The hexane layer after appropriate dilution with the same solvent was analysed for vinclozolin by GLC. In the experiment on the degradation of vinclozolin in mineral salts medium, a portion (1–2 ml) of the medium was extracted with an equal volume of hexane, dried over sodium sulfate (100 mg) and analysed by GLC. Extraction of residues and sample preparation were done in the laboratory in diffuse light.

2.6 Gas-liquid chromatography

Vinclozolin residues extracted in hexane were analysed in a Varian gas chromatograph model 3400 equipped with a ^{63}Ni detector and a metal column (2 m length, 3 mm OD) packed with 3% OV-17 on Chrom WHP 80/100 mesh. The operating conditions were as follows: carrier gas (argon + methane, 95 + 5 by volume) flow, 20 ml min^{-1}; injector temperature 240 °C; column temperature 220 °C; detector temperature 240 °C. Under these conditions, the retention time of vinclozolin was 2.8 min. Recovery of vinclozolin from soil and water samples following the extraction and analytical procedures employed was 96 (± 2.6)% and the limit of detection was 0.05 μg of the compound. All the results presented are the mean of duplicate observations.

2.7 Measurement of Eh and pH

Soil samples (40 g) placed in 100-ml beakers were flooded with 50 ml sterile distilled water and incubated at room temperature. At 10 days after flooding, 1 ml aqueous suspensions of vinclozolin WP was added to each sample to provide a final concentration of 50 μg AI g^{-1} soil. At periodic invervals, the redox potential of duplicate soil samples was measured by inserting a combined platinum-calomel electrode (Barnant Co IL, USA) into the soil and measuring the potential difference in mV.[12] Immediately after the measurement of the redox potential, the pH of the soil was measured with a portable pH meter (Philips model PW 9424, Philips Analytical, Cambridge, UK).

Table 3. Changes[a] in the redox potential (Eh) and pH of the three soils under flooded condition

	Soil					
	Alluvial		Kari		Pokkali	
Days of flooding	Eh[b]	pH	Eh	pH	Eh	pH
0	250	6.16	279	3.41	248	5.51
10	−163	6.51	−85	5.40	−159	6.78
20	−183	6.70	−127	5.88	−203	7.23
30	−202	6.80	−162	6.40	−221	7.27
40	−219	6.87	−175	6.56	−247	7.31
50	−221	6.92	−180	6.61	−284	7.30
60	−242	7.18	−202	6.72	−292	7.32

[a] Mean of duplicate observations.
[b] mV.

3 RESULTS AND DISCUSSION

3.1 Persistence of vinclozolin under non-flooded and flooded conditions

The persistence of the commercial formulation of vinclozolin was studied in three soils under both non-flooded and flooded conditions (Table 2). The amount of vinclozolin remaining in the soil was plotted on a log scale against the time of incubation.[2] The degradation of vinclozolin in all three soils followed a first-order reaction as the plots yielded straight lines based on the equation

$$C = C_0 e^{-kt}$$

where C is the concentration of the fungicide remaining in the soil after time t, C_0 is the initial concentration and k is the first-order kinetic constant. The half-life ($t_{1/2}$) values (Table 2) obtained from these plots indicated that the fungicide, in general, appears to be less persistent in all the three soils under flooded conditions than under non-flooded conditions. Among the three soils under non-flooded conditions, degradation of vinclozolin was the least in Kari soil. A simple linear correlation analysis between $t_{1/2}$ and different soil properties under non-flooded conditions indicates a significant relationship with pH ($r = 0.997$) and electric conductance ($r = 0.998$) of the soils. Vinclozolin degrades slowly in soils with low pH (5.0–5.5),[9] and is stable under acidic conditions.[13,14] Likewise, several pesticides have been reported to be increasingly persistent under saline conditions.[15] Increasing salinity is inhibitory to growth and activity of micro-organisms.[16] This would explain the increased persistence of vinclozolin in Kari soil.

Under flooded conditions, the pH of the soils increased to near neutrality within a few days of flooding (Table 3). All the soils used in this study were microbially active, as indicated by a sharp drop in the redox potential and simultaneous increase in pH following flooding. Thus, the near-neutral pH and high microbial activity could be the reasons for faster degradation of vinclozolin under flooded conditions. Detection of 3,5-dichloroaniline in the soils indicated that hydrolysis is the major pathway of degradation of the fungicide.[6,9]

3.2 Enhanced degradation of vinclozolin in an alluvial soil following repeated applications

The development of enhanced degradation of vinclo-

Table 2. Persistence of vinclozolin in an alluvial soil under non-flooded and flooded conditions

	Vinclozolin recovered (μg g^{-1} soil)[a,b]					
	Alluvial		Kari		Pokkali	
Incubation (days)	Non-flooded	Flooded	Non-flooded	Flooded	Non-flooded	Flooded
0	47.4	40.3	47.1	42.7	45.6	41.0
10	37.1	27.7	45.8	36.4	40.5	35.4
20	28.1	12.4	36.6	24.4	33.6	21.6
30	19.7	9.4	30.0	19.2	26.8	6.9
40	13.0	4.6	24.1	5.0	19.2	2.6
50	10.4	2.2	19.2	3.6	13.2	1.1
60	7.5	1.3	17.3	2.1	11.2	tr[c]
K	0.0316	0.0584	0.0184	0.0545	0.0251	0.0773
r[d]	0.998	0.996	0.991	0.971	0.988	0.977
$t_{1/2}$ (days)	21.91	11.85	37.63	12.70	27.58	8.96

[a] Initial concentration 50 μg g^{-1} soil.
[b] Mean of duplicate observations.
[c] tr - trace.
[d] Significant at $P < 0.01$.

Table 4. Degradation of vinclozolin in a mineral salts medium inoculated with suspension of soil from unplanted pots maintained under non-flooded or flooded conditions and retreated with vinclozolin

	Vinclozolin recovered[a,b] ($\mu g\ ml^{-1}$)								
				Pretreated with vinclozolin					
		Untreated		*2nd application*		*3rd application*		*4th application*	
Incubation (days)	*Uninoculated*	*Non-flooded*	*Flooded*	*Non-flooded*	*Flooded*	*Non-flooded*	*Flooded*	*Non-flooded*	*Flooded*
0	9.8 (± 0.1)	6.9 (± 0.4)	6.7 (± 0.1)	6.8	6.6 (± 0.1)	7.0 (± 0.4)	6.8 (± 0.7)	6.0 (± 0.2)	5.8 (± 0.1)
3	ND[c]	ND	ND	ND	ND	1.9 (± 0.1)	2.2 (± 0.1)	1.9 (± 0.3)	1.4 (± 0.3)
5	2.6 (± 0.4)	2.4 (± 0.2)	2.0 (± 0.4)	1.9 (± 0.8)	1.8 (± 0.1)	1.9 (± 0.2)	1.7	0.9 (± 0.1)	0.8
8	ND	ND	ND	ND	ND	1.2 (± 0.7)	1.0 (± 0.3)	0.5 (± 0.2)	0.2 (± 0.1)
10	1.4 (± 0.1)	1.4 (± 0.2)	1.3 (± 0.1)	0.9 (± 0.3)	0.8 (± 0.3)	0.5 (± 0.4)	0.3	0.1	0

[a] Initial concentration 10 μg ml^{-1} mineral salts medium.
[b] Mean of two replicate observations (±mean deviation).
[c] ND = Not determined.

Table 5. Degradation of vinclozolin in a mineral salts medium inoculated with suspension of soil from planted pots maintained under non-flooded or flooded condition and retreated with vinclozolin

	Vinclozolin recovered[a,b] ($\mu g\ ml^{-1}$)								
				Pretreated with vinclozolin					
		Untreated		*2nd application*		*3rd application*		*4th application*	
Incubation (days)	*Uninoculated*	*Non-flooded*	*Flooded*	*Non-flooded*	*Flooded*	*Non-flooded*	*Flooded*	*Non-flooded*	*Flooded*
0	9.8 (± 0.1)	6.6 (± 0.1)	6.6 (± 0.1)	6.8 (± 0.2)	6.4 (± 0.4)	6.7 (± 0.3)	6.6 (± 0.1)	6.2 (± 0.3)	6.0 (± 0.3)
3	ND[c]	ND	ND	ND	ND	2.2 (± 0.1)	2.1	2.7 (± 0.2)	1.0
5	2.4 (± 0.4)	2.5	2.1 (± 0.2)	2.0 (± 0.8)	1.6 (± 0.2)	2.2 (± 0.1)	1.5 (± 0.6)	1.2 (± 0.2)	0.4 (± 0.1)
8	ND	ND	ND	ND	ND	1.4 (± 0.4)	0.9 (± 0.4)	0.2	0
10	1.4 (± 0.1)	1.8 (± 0.3)	1.9 (± 0.3)	0.9 (± 0.2)	0.7 (± 0.1)	0.6 (± 0.3)	0.2	0	0

[a] Initial concentration 10 μg ml^{-1} mineral salts medium.
[b] Mean of two replicate observations (±mean deviation).
[c] ND = Not determined.

zolin was examined after repeated addition of a commercial formulation of vinclozolin to non-flooded or flooded alluvial soil under greenhouse conditions. No accelerated degradation was observed after the first or even second addition of the fungicide. After the third application, vinclozolin degradation was faster, especially under flooded conditions (Table 4). During the same period, there was some loss of the fungicide from the uninoculated control and from the medium inoculated with suspensions from untreated soil, possibly due to chemical hydrolysis.

As in the unplanted pots, accelerated degradation of vinclozolin developed also in the planted pots (Table 5). However, unlike unplanted soil, enhanced degradation in planted soil was evident from the second application onwards and was intense after the fourth application. Interestingly, soil planted to rice and maintained under flooded conditions exhibited more clear-cut enhancement of vinclozolin degradation. Enhanced degradation of vinclozolin in laboratory and field soils following their repeated additions has been demonstrated earlier.[9] However, what was interesting in the present study is the role of plants in influencing the development of enhanced degradation of the fungicide. Flooded soil planted to rice is physically, chemically and microbiologically more active than its non-flooded unplanted counterpart.[17] Rice plants are known to exert a positive influence on soil microbial activity through the transport of oxygen to the root region as well as by the release of root

Table 6. Persistence of vinclozolin in a mineral salts medium inoculated with sterile or non-sterile suspension of soil retreated with vinclozolin

	Vinclozolin recovered[a,b] ($\mu g\ ml^{-1}$)		
Incubation (days)	*Uninoculated*	*Inoculated with sterile soil suspension*	*Inoculated with non-sterile soil suspension*
0	9.2 (± 0.4)	8.8 (± 0.3)	8.9 (± 0.2)
3	2.5 (± 0.2)	2.8 (± 0.4)	2.4 (± 0.3)
5	2.2 (± 0.4)	2.1 (± 0.5)	0.8 (± 0.2)
10	1.6 (± 0.3)	1.4 (± 0.5)	0

[a] Initial concentration 10 μg ml^{-1} mineral salts medium.
[b] Mean of duplicate observations (±mean deviation).

exudates.[18] Enrichment of vinclozolin-degrading micro-organisms has been considered to be the cause of enhanced degradation of the fungicide.[6] Thus high microbial activity in a flooded soil planted to rice could be the reason for enhanced degradation of vinclozolin following its repeated application.

The role of micro-organisms in the degradation of vinclozolin was investigated in sterile vs non-sterile soil suspensions of flooded soil showing enhanced degradation of vinclozolin. Vinclozolin disappeared faster in mineral salts medium inoculated with non-sterile soil suspension than in medium inoculated with soil suspensions sterilized by autoclaving (Table 6). In studies on microbial conversion of vinclozolin, the fungicide was shown to be hydrolysed producing persistent chlorinated metabolites including 3,5-dichloroaniline.[6] In the present study also 3,5-dichloroaniline was detected as a metabolite in the degradation of vinclozolin. Enhanced degradation of the organochlorine insecticide hexachlorocyclohexane was reported following repeated application of the parent compound[3] or its metabolite[4] to flooded soil planted to rice. This study demonstrates the development of enhanced degradation of vinclozolin, a chlorine-containing dicarboxymide fungicide, after two or three additions of the parent compound to a flooded soil.

ACKNOWLEDGEMENTS

We thank Dr K C Mathur, Director, for permission to publish this work. The work was supported, in part, by a research grant from Department of Science & Technology, Government of India to the senior author. Ms S Padhi was supported by a fellowship from University Grants Commission, New Delhi.

REFERENCES

1 Sethunathan N, Rao VR, Adhya TK and Raghu K, Microbiology of rice soils – a review. *CRC Crit Rev Microbiol* **10**:125–172 (1983).
2 Alexander M, *Biodegradation and Bioremediation*, Academic Press Inc, San Diego (1994).
3 Bhuyan S, Sahu SK, Adhya TK and Sethunathan N, Accelerated aerobic degradation of γ-hexachlorocyclohexane in suspensions of flooded and non-flooded soils pretreated with hexachlorocyclohexane. *Biol Fertil Soils* **12**:279–284 (1992).
4 Bharati K, Padhi S and Adhya TK, Accelerated biodegradation of γ-hexachloroclohexane (γ-HCH) in a flooded alluvial soil pretreated with γ-HCH or its metabolite 1,2,4-trichlorobenzene (TCB). *Bull Environ Contam Toxicol* **60**:858–863, (1998).
5 Spencer EY, *Guide to the Chemicals Used in Crop Protection*, 7th edn, Publication No. 1043, Agriculture Canada Research Branch, Ottawa, Canada (1982).
6 Golovleva LA, Finkelstein ZI, Polyakova AV, Baskonov BP and Nefedoya MY, Microbial conversion of the fungicide vinclozolin. *J Environ Sci Health* **B26**:293–307 (1991).
7 Sumida S, Yoshihara R and Miyamoto J, Degradation of 3-(3,5-dichlorophenyl)-5,5-dimethyloxazolidine-2,4-dione by plants, soil and light. *Ag Biol Chem* **37**:2781–2790 (1973).
8 Szeto SY, Burlinson NE, Rahe JE and Oloffs PC, Persistence of the fungicide vinclozolin under laboratory conditions. *J Ag Fd Chem* **37**:529–534 (1989).
9 Walker A, Brown PA and Entwistle AR, Enhanced degradation of iprodione and vinclozolin in soil. *Pestic Sci* **17**:183–193 (1986).
10 Panda S, Sharmila M, Ramanand K, Panda D and Sethunathan N, Persistence of hexachlorocyclohexane isomers and carbofuran applied to surface and sub-surface layers of a flooded soil. *Pestic Sci* **23**:199–207 (1988).
11 Walker A, Enhanced degradation of iprodione and vinclozolin in soil – a simple colorimetric test for identification of rapid-degrading soils. *Pestic Sci* **21**:233–240 (1987).
12 Pal SS, Barik S and Sethunathan N, Effects of benomyl on iron and manganese reduction and redox potential in flooded soil. *J Soil Sci* **30**:155–159 (1979).
13 Melkebeke G, De Spiegeleer F, Strubaut W and Dejonckheere W, A kinetic study of the chemical hydrolysis of the fungicides iprodione, vinclozolin and metalaxyl. *Med Fac Landbouw Rijksuniv Gent* **51**:319–324 (1986).
14 Szeto SY, Burlinson NE, Rahe JE and Oloffs PC, Kinetics of hydrolysis of the dicarboximide fungicide vinclozolin. *J Ag Fd Chem* **37**:524–529 (1989).
15 Adhya TK, Wahid PA and Sethunathan N, Persistence and biodegradation of selected organophosphorus insecticides in flooded versus non-flooded soils. *Biol Fertil Soils* **4**:36–40 (1987).
16 Zahran HH, Diversity, adaptation and activity of the bacterial flora in saline environments. *Biol Fert Soils* **25**:211–223 (1997).
17 Yoshida T, Microbial metabolism in flooded soils, in *Soil Biochemistry*, vol. 3, ed by Paul EA and McLaren AD, Marcel Dekker, New York. pp 83–122 (1975).
18 Watanabe I and Furusaka C, Microbial ecology of flooded soils, in *Advances in Microbial Ecology*, vol 4, ed by Alexander M, Plenum Press, New York. pp 125–168 (1980).